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A mutant affecting wing pattern in *Parnassius apollo* (Linne) (Lepidoptera Papilionidae)

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Abstract. A mutant affecting wing pattern has been observed repeatedly and over a large number of years in a population of *P. apollo* from the upper Durance basin in France. It is dominant and morphologically modifies the postcellular region of forewings and the posterior part of hindwings, inducing a mask-like design in the former and obliterating the second eyespot in the latter. The frequency of the mutant in the population was 1 to 2% in the late 1970's. It has markedly decreased since.

Introduction

Scores of aberrations have been described in *Parnassius apollo*. However, no genetic work has been carried out although breeding of this species has been practised for some time. The main difficulty is obtaining mating in captivity. One of us has mastered the problem by hand-pairing. The method may permit production of a practically indefinite number of successive generations and thus genetic experimentation. The present paper, the first of a series with such experiments, involves a very spectacular aberration.

Materials and methods

Ova of either field-collected or bred females are obtained by placing females singly in a plastic-gauze cage of *ca* 1 liter (this device will be described with more details in a later paper). Oviposition is induced either by filtered sunshine or by a 60-100W incandescence bulb placed 20 to 50cm from the cage. In all cases, overheating *must* be carefully avoided. Ova are deposited singly or in small batches upon 1) a cellulose towel placed on the bottom of the cage, 2) foodplant fragments (*Sedum sp, Sempervivum*) which are not necessary to elicit laying, or 3) the cage walls. Regular feeding, once or twice a day, by a honey-water mixture (1:10) is essential. We have observed that old, almost exhausted wild females will recommence laying if carefully fed for several days. In all cases, it is preferable to allow the females to lay for only a limited period (1-2 hours per day) and to keep them quiescent in a cool, shady place the rest of the time. Under these conditions, females can live 3 weeks or more and lay between 100 and 200 ova.

The ova generally diapause and are best refrigerated for at least 2 months at 0-4°C. However, a small portion of ova (1 to 5% in French populations, but much more in Spanish ones) hatch immediately and may be reared to adults by the end of summer (this observation implies the possibility of a potential partial second generation under natural conditions).

For breeding larvae, it is important to maintain a condition of cool or even cold air while using a heat radiating light: this condition can be satisfied using either sunshine or artificial light. It is possible to greatly accelerate caterpillar growth rate by continuous lighting, pupae being obtained within 10 days. Foodplants are various species of *Sedum*, according to their availability. *S. album*, of low vegetation, is especially convenient for starting young caterpillars, which do not spin silk and are consequently unable to climb over elevated plants. Some cultivated species are refused (*e.g. S. acre*) and may be toxic. The broods must be well ventilated, covering with gauze is unadvisable. Glass or plastic pans with appropriately high walls are convenient, since the walls are impassable barriers to the non-climbing caterpillars. Palik's method (1980), using cellophane walls, is more sensitive to use and can cause trapping of young larvae at the base of the plastic sheet. The offspring of a mutant female was lost in this way in 1980, which delayed the completion of the present study until 1984.

Copulation is easily obtained between bred individuals. The butterflies may pair freely even in a small cage (for instance a 50cm side cube), provided there is sufficient sunshine. However, hand-pairing affords the most reliable control of partner choice. We used Clarke and Sheppards's method (1953). Although *Parnassius* are markedly more difficult to pair than *Papilio*, success is generally complete when conditions are good and the operator skilled. Key factors are that males must be excited by sunshine and the females young. Although females can be kept ready for mating in a refrigerator at 4°C for several days, the freshest are best. Pairing lasts several hours (the couple is left still for this time under attenuated light). One male is capable of fecundating at least three females. In the first mating, a large, well formed sphragis is secreted; in the second one, this appendage is rudimentary and is absent in the third.

In all cases, however, fecundation is complete. We recall here that the sphragis is *not* a "laying pouch" as once stated, but a true "chastity belt", precluding further fecundation. The presence of fecund females with no sphragis or with a rudimentary one in the field is a strong indication that males can practise several successive matings. It is also possible that a female could be fecundated at least twice, first by an old male no longer able to secrete a sphragis and again by a young male. Such an event would be exceptional yet possible to check by counting the spermatophores present in the *bursa copulatrix*.

Results

FIELD OBSERVATIONS: For obvious reasons, we will not give the exact location where mutant individuals have been observed. It generally lies in the upper Durance basin, in french southern Alps. The habitat is a large set of rather smooth, sunny barren slopes, intermingled with mowed meadows, at *ca.* 1800m elevation. Few trees are present, a condition quite probably due to forest destruction by man. The substrate is essentially formed by moraines and screes, with some thalwegs, not very accentuated, and a small stream. *Sedum* and *Sempervivum* are quite abundant and provide food for *Parnassius apollo*. The Parnassians themselves are very abundant over a large area. We carried out a mark-release-recapture study over a precisely defined small area of the flight locality. This experiment allowed us to estimate the population flying upon this area to 400-500 individuals (Napolitano, Cooke and Descimon, in preparation). The total area of the locality is much larger and extrapolation of the data allows to estimate the order of magnitude of the population being at least 10,000. By direct behavioral observation, it was seen that the butterflies move freely from one point to another over distances of at least one kilometer, as confirmed by the capture of marked individuals at distances of this order from their previous marking area. However, inhospitable zones circumscribe flight areas to some extent. In such inhospitable zones, individuals are casually seen, but are much scarcer by comparison. Other high density flight areas exist some kilometers away from the main one, but they are markedly smaller.

The first aberrant individuals were taken August 9, 1977, with three taken on one day. Only later on did we realize that, since the aberration was recurrent, it was probably due to mutation and that its frequency was worth further investigation. Still later, we discovered in the correspondence of the senior author a letter from Lucien Jean, who mentioned the capture of an "aberrant apollo" by another collector, Mr. Dreano, in the same locality. The letter was accompanied with a color slide which allowed us to verify that the female aberrant collected by Mr. Dreano belonged to the same type we found. This specimen had been captured around 1975.

The population has been followed regularly to 1981 and less intensively since 1982. We attempted to count all individuals seen to obtain a gross estimation of the mutant frequency (Table I). In most cases, counts were made by direct sighting, without marking correlation, so results must be considered approximate. At face value the frequency of the mutant decreased from ca. 2% to ca. 0.5% in five years. However, the 1985 capture of a normal female which produced mutant offspring indicated it had been mated by a mutant male and that the gene was still present in the population at that time.

A substantial fraction of the mutant individuals was secured, in particular 3 females for laying. Foolishly, we made the faulty assumption that the mutation was recessive, which would have implied that removing the thus supposedly homozygous individuals was not detrimental. This supposition was quite unfortunate, as we will see further.

It is worth noting that, when mutants were observed, they generally were in a group of 2-5 individuals flying in a restricted "pocket" surrounded by areas where none was to be found. It seems that this pocket correspond to the laying area of the mother female. Sometimes,

Table I. Number and percentages of "*Zorro*" mutant vs. "normal" phenotypes of *P. apollo*.

Year	Numbers of *P. apollo* observed		Percentage
1977	"normal" phenotype	"*Zorro*" mutants	
1977	*ca* 600*	12 (8 ♂, 4 ♀)	2.0
1978	*ca* 800*	14 (12 ♂, 2 ♀)	1.7
1979	1184**	7 (6 ♂, 1 ♀)	0.6
1980	136**	1 ♀	0.3
1981	*ca* 300*	0	0.0
1983	ca 200*	1 ♂	0.5
1985	*ca* 200*	1 ♂***	0.5
1987	*ca* 150*	0	0.0

* Individuals counted but not marked; in this case, we applied a correction coefficient keeping in account multiple captures and deduced from marking-releasing experiments.
** Individuals marked before being released.
*** Individual not observed, but existence deduced from the offspring of a "normal" female.

mutants were observed flying slightly more awkwardly than the normal butterflies and, in some cases, the degree of wing damage indicated they were less sturdy. Behavior was unaffected, but phenotype modification was discernible during flight to an experienced eye from a distance. When at rest in the absence of sunshine, *Parnassius* display a characteristical protective behavior. They open their wings in a horizontal plane and reveal their posterior eyespots. This display is accompanied by a kind of stridulation obtained by brushing the ventral face of hindwings with posterior legs (Descimon, 1965). The resultant noise resembles bruising silk and is perceptible to human ear from at least 1 meter. This behavior seems to occur in all species of *Parnassius*, including both *P. mnemosyne*, in which the hindwings do not have red eyespots, and the very ornate blue and red Himalayan species (F. Michel, pers. comm.). The mutants also display this behavior, but it's effect is entirely different to human observer's eye: attention is drawn from the hindwings, where the posterior eyespot is missing, to the forewings with their striking mask-like design.

BREEDING. In 1983, a male was secured and handpaired with a virgin female from the Lubéron (Vaucluse, France). A 1:1 segregation appeared in the offspring and, since the mutation had never been observed among thousands of butterflies in the Lubéron population, the mutation must be dominant. The gene was subsequently introduced into other stocks, including those from the Mercantour (Alpes Maritimes, France) and the Causse du Larzac (Aveyron, France). Further, a new mutant strain, isolated from the original Durance locality, was recovered by chance. As already mentioned above, a normal female was collected amongst a group of normals. This female yielded mutants in a 1:1 ratio, which indicated that it had been fecundated by a heterozygous mutant male. In all mutant x normal crosses a 1:1 segregation appeared (table II). The only heterozygote x heterozygote cross (table II No.6) yielded a high proportion of mutant individuals. This result is puzzling. Indeed, the other crosses do not depart significantly from a 1:1 ratio, which precludes considering a superiority of heterozygotes over normal homozygotes. In No.6 cross, the excess of mutant phenotypes is not only too high to allow considering the possibility of homozygous mutant lethality (2:1 ratio, $X^2 = 9.6$, $p < 0.01$), but even to fit with the expected 3:1 ratio ($X^2 = 5.4$, $p < 0.02$). We, however, cannot draw conclusions from a single cross. Actually, the genetic composition of this brood was fairly heterogenous, which could give rise to "hybrid breakdown" phenomena and distort the ratios. In particular, we have indications that the Causse du Larzac population is peculiar; for instance, its ova are on the average twice as heavy as those from Alpine populations. The concerned cross was the only laboratory-bred of a series of identical parentage which was used to make an experiment of founding an artificial population of *P. apollo* on the Sainte Baume mountain, where it is absent. Now, this experiment, which involved the deposition of 1,000 ova upon favorably

Table 2. Results of crosses with *"Zorro"* mutant of *Parnassius apollo*.

Number of the cross	Parents	Offspring "normal"	*"Zorro"*
1	♂: *"Zorro"* from *"Zorro"* ♀ x "normal" ♂ from Mercantour ♀: wild, normal, Briançon	21 (9 ♂, 12 ♀)	24 (9 ♂, 15 ♀)
2	♂: wild, "normal", Causse du Larzac ♀: *"Zorro"* from *"Zorro"* ♀ x wild "normal" ♂, Lubéron	16 (7 ♂, 9 ♀)	17 (10 ♂, 7 ♀)
3	♂: *"Zorro"* from a "normal" ♀ caught in the wild ♀: "normal" from all-normal F_1 from the original locality	40 (14 ♂, 16 ♀)	39 (19 ♂, 10 ♀)
4	♂: wild, "normal", Mercantour ♀; *"Zorro"* from 3	7	7
5	♂: wild "normal", Lubéron ♀: *"Zorro"* from 3	7	12
6	♂: *"Zorro"* from 2 ♀: *"Zorro"* from 2	2	28

sited foodplants, has been unsuccessful. Moreover, the mortality in the brood was high (only 30 adults from 110 ova), in spite of having been pampered. More experiments are needed but, unfortunately, they are at present impossible.

MUTANT GENE EXPRESSION AND VARIATION. The average mutant phenotype is represented on figure 1 (A: male, B: female). On the forewings, it is the region posterior to vein 7 which is modified: the discal spot is elongated distally into a point following vein 7. Basally, this spot shows a tendency to be connected to the median (discocellular) spot of the cell by two black streaks following the anterior and posterior limits of the cell. The marking gives mutant butterflies a striking aspect, as if they bear a black mask. The postdiscal row of spots is shifted distally and partly obliterated in 5-6 and 6-7 intervals. No obvious modification is to be noticed in 2-3 and 3-4 intervals, but the spot of the 1b-2 interval, very characteristic of the species, is conspicuously shrunken and divided into two parts by 1c rudimentary vein. On the hindwings, the anterior eyespot is absolutely unaffected, while the posterior one, which lies in

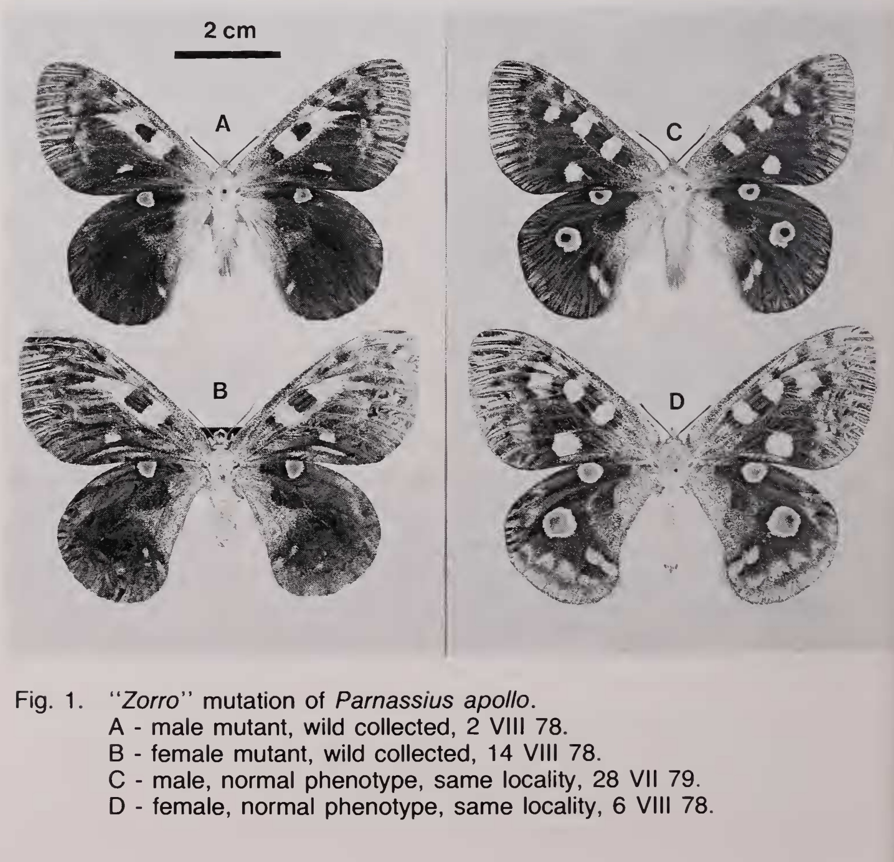

Fig. 1. "*Zorro*" mutation of *Parnassius apollo*.
A - male mutant, wild collected, 2 VIII 78.
B - female mutant, wild collected, 14 VIII 78.
C - male, normal phenotype, same locality, 28 VII 79.
D - female, normal phenotype, same locality, 6 VIII 78.

3-4 and 4-5 intervals, is extremely modified. It is dissociated into two parts, as if a factor following vein 4 inhibited the formation of the eye-spot pattern. The series of anal spots in 1b, 1b-2 and 2-3 is diminished but still present. Premarginal black scales lunules and marginal hyaline band are slightly displaced basally.

It is rather obvious that such a remarkable mutation deserves a name. Here we meet with a problem which has not been considered seriously for Lepidoptera. In species such as *Drosophila melanogaster*, which has not been plagued by a crowd of aberrational names given by mere "curios" collectors, matters are clear. Mutational names are indispensalbe working tools, with simple and clear terminological rules. In all animals, the practice of giving latin names to any infrasub-specific form has been rejected by the International Commission of Zoological Nomenclature. We believe that this does not preclude using non-linnean names which would follow the rules of genetic nomenclature in the case of variants which have in fact been studied genetically. The controversial situation arises in the case of "classically named aberrations" or morphs which later prove to be mutants. At first sight, it seems advisable to retain the old names. However, some cases would be quite puzzling; for instance, for the white female of *Colias croceus*, should we use the old name of "*Helice*" given by Hübner or, following the American authors, the generic name of "*Alba*", which assume that all white female mutants are homologous? In any event, we propose here the *mutational* name of "*Zorro*" for the described variant of *P. apollo*. Names that we would have preferred, such as *mephisto* or *diabolicus*, have been already given to *Parnassius* variants or subspecies; otherwise, the selected name will recall that nomenclature need not be such a serious topic, after all...

Field-collected, as well as bred examples of "*Zorro*" display variability. We noted above this is not due to incomplete dominance. The argument is reinforced by the fact that the probability of occurrence of homozygous mutant individuals in natural populations is very low (practically equal to variant frequency, that is 2 to 0.5 percent). Moreover, when variation is observed within bred individuals, it must be due mainly to interaction of the mutant gene with its genetic background, since rearing conditions were kept relatively constant. Fig. 2 shows some of this variability; individual 1 is among the least accentuated and 2 among the most. There is also an interaction with sexual dimorphism. If venation is not markedly modified on forewings, some striking abnormalities may be observed on hindwings. In all cases, a rudimentary cell is present at the outer extremity of the cell between M1 and M2 normal veins, but much more spectacular modifications can also occur: the cell is open between M1 and M2; M1 is often branched and in some cases a complex system of supplementary cells is formed. These modifications are often assymetrical. Figure 3 gives an idea of these atypical vein patterns.

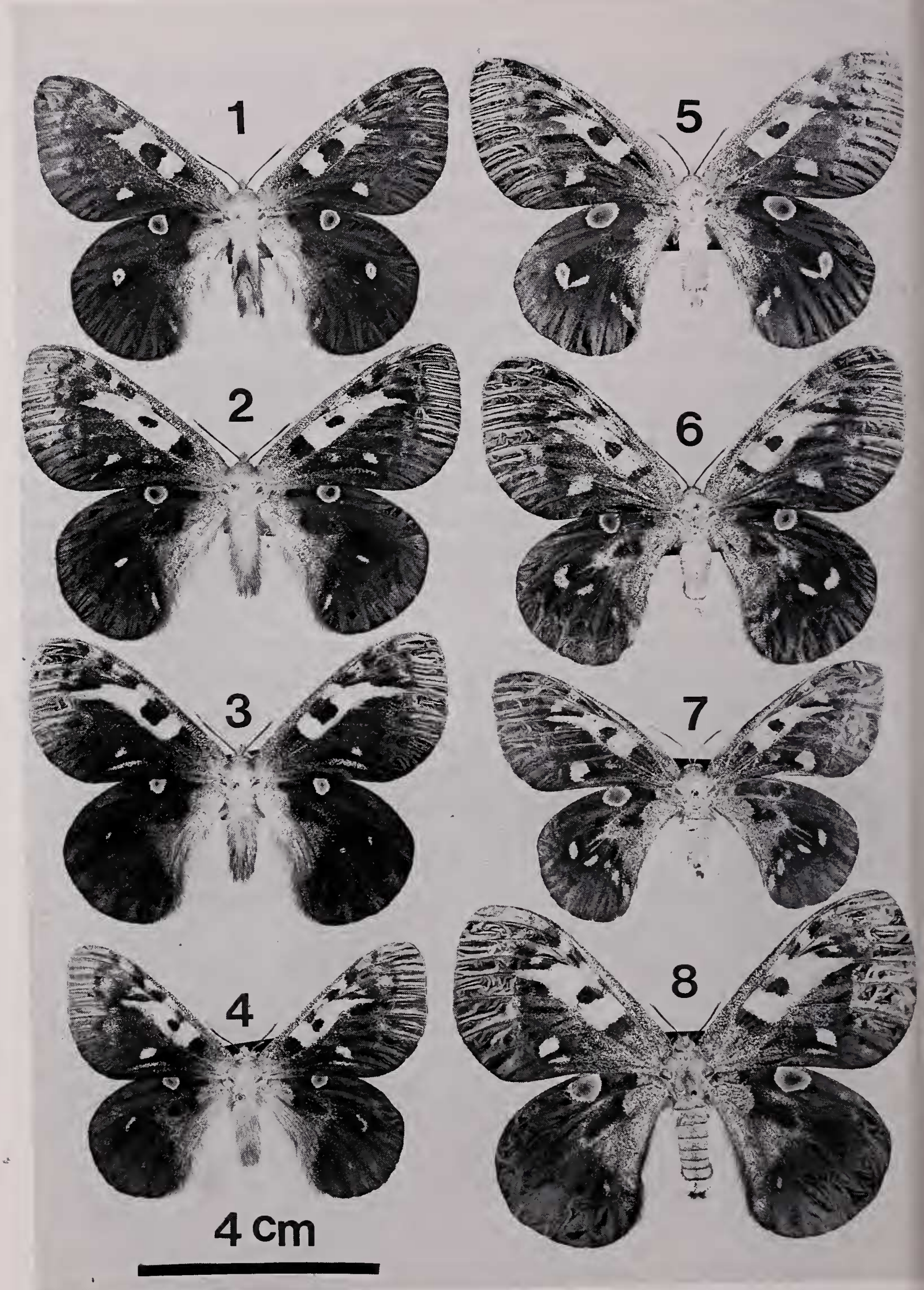

Fig. 2. Variation in the expression of "*Zorro*" mutation in *Parnassius apollo*.
1 - male, wild, 13 VIII 77.
2 - D°, D°, 27 VII 78.
3 - D°, D°, 14 VIII 78.
4 - D°, D°, 28 VII 79.
5 - Female, D°, 2 VIII 78.
6 - D°, D°, 10 VIII 77.
7 - D°, bred (Brood Number 1, see table II).
8 - D°, D° (Brood Number 2)

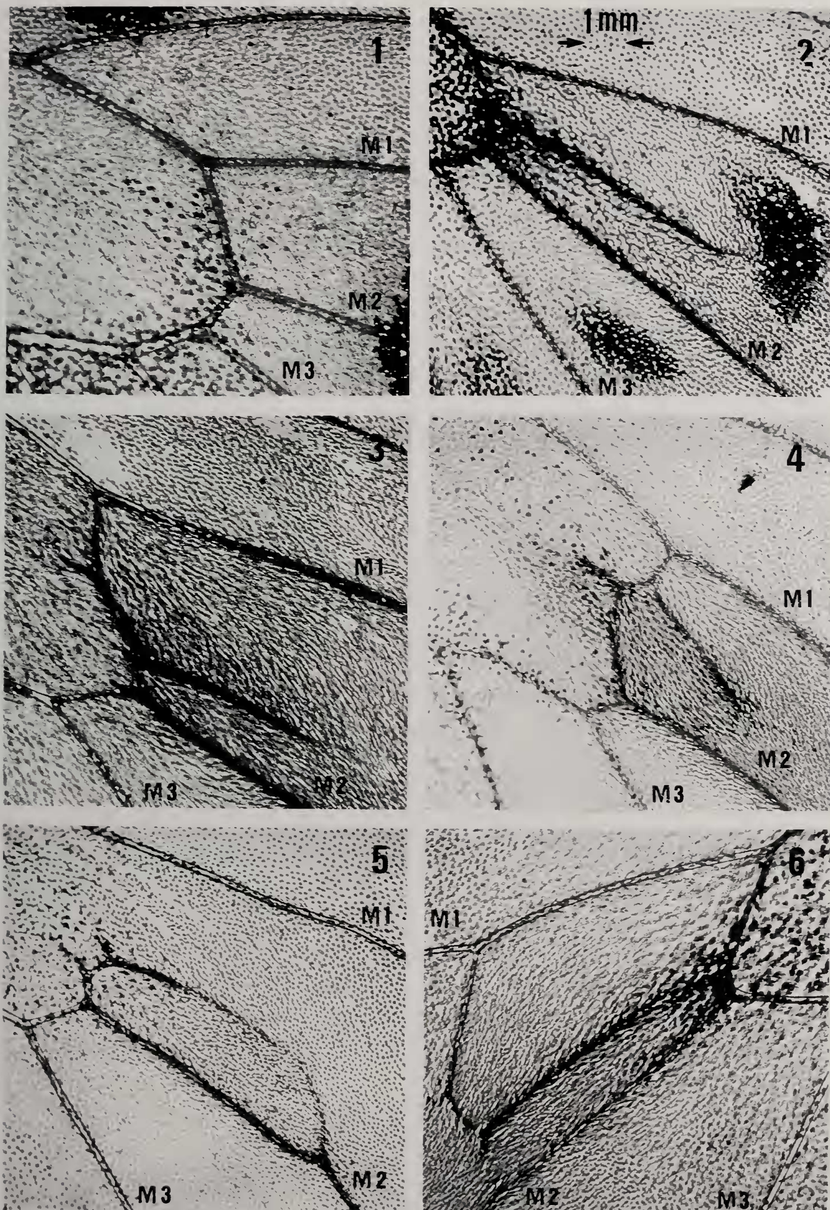

Fig. 3. Abnormalities of venation in "Zorro" mutation of *P. apollo*.

1. Normal female.
2. "*Zorro*" female (n° 6 of fig. 2), with supplementary distal vein.
3. D°, brood n° 6: supplementary distal vein plus intracellular rudimentary vein.
4. D°, male, brood n° 6: D°, with different branching.
5. D°, female, brood 5: supplementary cell with atrophy of normal cell closure.
6. D°, female, brood 5: supplementary distal cell.

All figures represent right hindwing in the region where median veins (M1, M2, M3) take rise from the cell, except n° 6, where it is left hindwing which has been photographed.

Discussion

From a morphogenetic point of view, "*Zorro*" is one of the most striking aberrations in *Parnassius*. A thorough survey of the previous described forms in available literature (*e. g.* Bryk, 1935, Eisner, 1966) did not reveal any close equivalent. It may be remarked that, in both pairs of wings, the mutation modifies only the posterior half, while the anterior one remains unaffected. Pattern and venation are most perturbated at the suture between the imaginal disk compartments which, according to the studies of Sibatani (1981), follows the axis of symmetry of the cell and the corresponding distal part of the wing. It is obvious that "*Zorro*" could provide a choice tool for the study of the development of wing pattern, using for example the methodology of Nijhout (1985). Unfortunately, it is probable that practical difficulties would render such a study rather difficult.

From the point of view of evolutionary genetics, the history of this mutation appears clear. It arose once, at one locus of one individual of the population. It is more difficult to understand how its frequency reached 1 or 2 percent. If we assume the size of the population, previously estimated to roughly 10,000, the original frequency must be around 0.5×10^{-4}. To reach the 10^{-2} frequency observed in 1977, the "*Zorro*" allele therefore must have been multiplied by 200. The simplest explanation would be to assume the population had been reduced to few individuals in at least one year, the mutation having been preserved by chance (or having appeared) during the time of the population contraction. Further, its frequency would have been amplified in parallel to the population increase. We can provide some observational support to this hypothesis: the senior author and his brother Robert Descimon have collected and observed butterflies very regularly in the region until the present time and *P. apollo* was noted as very scarce at the end of the 1960's. It is further noteworthy that the "*Zorro*" mutation was not observed in the locality where it was later discovered. Many *P. apollo* were seen during early 1960's.

Could selection have played a role in the variation of the frequency of the mutation? From 1977 to 1981 "*Zorro*" has obviously decreased. It is very unfortunate that we did not surmise that this aberration could be a dominant but postulated it was a recessive, with a gene frequency of about 0,14, providing the observed "homozygote" frequency of 2 percent. Under these conditions, we incorrectly believed that securing and killing some individuals was not detrimental. Actually, we introduced a massive selection coefficient, "destroying our own subject of study", according to the accurate expression of Dubois (1983). Fortunately, only a portion of the population was screened and the mutation was not eradicated in totality. The most distressing consequence of this thoughtless action is that we are now hindered from drawing conclusions. Would "*Zorro*" frequency have decreased anyway? It has been clearly observed that the mutants appear a trifle handicapped in flight activity.

At the larval stage, however, no disadvantage appears to exist; in one brood, an excess of mutants has been observed. The experiments should be repeated with a larger sample. The modification of wing pattern does not obviously impair its deterrent effect. To human eye, it is even more frightening! Therefore, we may not rule out the hypothesis that the mutation was slowly increasing its frequency when we clumsily intervened.

Although rather unusual, the dominance of "*Zorro*" seems to be best interpreted in terms of physiological genetics. The observed variation in expression does not seem related to overdominance but to the interference with the entire genotype. It would not be relevant to hypothesize that the mutation should have become dominant after a process of "evolution of dominance" (Ford and Sheppard, 1966), since none of the conditions for it appear here.

We have planned to "repair our fault" by breeding and releasing mutant individuals (with, of course, no mixture with foreign strains) into the locality. To do so, we would introduce yet another perturbation into the population. The best would be to proceed but with seriously controlled and monitored conditions. Only accidental difficulties have delayed this operation. We also intend to undertake again experiments of creating artificial populations like the one previously mentioned, which has been probably unsuccessful. Such attempts (which are debatable if not carefully designed) have proven successful and quite instructive in some cases (Descimon, 1976, Holdren and Ehrlich, 1981).

Cases of decreasing mutation frequency in natural populations by collecting are already known. The most striking is probably the "*honnoratii*" form of *Zerynthia rumina* in the region of Digne (Alpes de Haute Provence, France). The problem, which has elicited some row in the local press (with ridiculously exaggerated considerations, especially about the prices fetched on butterfly market) led to the promulgation of a law forbidding all insect collecting in the concerned department. Notwithstanding the inadequacy of enforcing the law, it is almost certain that it has improved the chance of maintaining the mutant gene. *Z. rumina "honnoratii"* has been observed in recent years (P. Bonnet, pers. comm.).

We believe that, for "*Zorro*" as for "*honnoratii*", the best protection is to breed and distribute them to collectors, who would be deterred from painstakingly seeking for them in nature. Further, lowering the venal value would render the "black market" less likely. One would pass from "hunting and gathering" to "farming". We strongly suggest "desperate hunters" not only search for *new* aberrations, but breed them, obtaining at the same time not only fine collection items, but genetic information. Such a practice has been frequent for some time in England - it must be generalized.

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